Apoptotic and necrotic cell death in cochlea treated with ototoxic chemicals

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Abstract

Background: Cell death can occur by apoptosis or necrosis. Apoptosis, an orchestrated intracellular death program, is triggered by a wide range of stimuli that regulate a network of apoptosis-related proteins that leads to the removal of damaged or excess cells. The major morphological characteristics of apoptosis are blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation and chromosomal DNA fragmentation. In contrast, necrosis is triggered by factors that cause cell swelling, membrane rupture and the uncontrolled release of the cell’s contents. We identified cochlear cells undergoing apoptosis and necrosis in vitro and in vivo after treatment with several ototoxic drugs. Cochlear explants were treated with gentamicin (0.5 mM) for 24 h or cisplatin (50 µM) for 48 h. In adult rats, acute cell death was investigated after co-administration of kanamycin (KM, 800 mg/kg, I.M.) plus ethacrynic acid (EA, 50 mg/kg, I.V.); chronic cell death in adult rats was investigated by treating adult rats for 9 d with daily injection of KM (800 mg/kg, I.M.).

Methods: Cochlea hair cell death was identified using an Apoptosis-Necrosis Quantification Kit. Apoptotic cells were identified by annexin V conjugated with FITC (green fluorescence) which specifically binds to the phosphatidylserine exposed on the outer membrane leaflet. Necrotic cells were identified by labeling with ethidium homodimer III (EthD-III, red fluorescence), a highly charged nucleic acid probe. Healthy cells were labeled with the membrane-permeant blue fluorescent DNA dye, Hoechst 33342.

Results: In acute KM/EA damage, apoptosis was more common than necrosis. In contrast, during chronic damage with KM injection for 7d necrosis was more common than apoptosis.

Conclusions: Chronic cell death from KM can occur either by an apoptosis or necrosis. Potential intervention strategies to promote hair cell survival will likely be most effective during KM/EA-induced apoptosis provided that the intervention is potent and occurs early enough to block the cell death cascade.

Methods

Experimental Models

- **Cisplatin or gentamicin treatment in vitro**
  - Cochlear organotypic cultures were treated with 10 µM cisplatin for 48 h or 500 µM gentamicin for 24 h.
- **Acute damage in vivo with KM and EA**
  - Adult rats were treated with EA (50 mg/kg, i.v.) plus KM (800 mg/kg, i.m.) and sacrificed 24 h later.
- **Chronic damage in vivo with KM**
  - Adult rats were treated with KM (800 mg/kg x 8 d, i.m.) and sacrificed 24 h after the last injection.

Apopotic, Necrotic and Healthy Cell (ANH) Labeling Kit

The “Apopotic & Necrotic & Healthy Cells Quantification Kit” (Biotium, Inc.) was used to identify apoptotic, necrotic and healthy cells.

Cochlear explants were labeled with working solution in the ANH kit for 60 min and then fixed with 10% formalin for 2 h. For adult rats, animals were anesthetized, the middle ear cavity opened and ANH working solutions in the kit perfused into the cochlea for 60 min. Afterwards, the cochlea was perfused with 10% formalin in PBS.

Cochlear surface preparations and observations

After fixation, the cochlear basilar membrane was micro-dissected out from the bony spiral plate of the modiolus. The basilar membrane was mounted in antifade solution on glass slides, and examined under a confocal microscope.

Results

In Vitro Cochlear Cultures

Fig. 1A z-radial image: Normal organ of Corti cultured for 48 h; only blue healthy cells in organ of Corti.

In Vivo Acute KM/EA Damage

Fig. 2 z-radial image: Control (A-D) Only blue healthy cells present, no apoptotic or necrotic labeling KM/EA (E-F) Both apoptotic and necrotic signaling present in inner hair cells (IHC) and outer hair cells (OHC). Intact supporting cells in blue.

In Vivo Chronic KM Damage

Fig. 3 surface image organ Corti Control (A-D) No apoptotic or necrotic cells in Control; only blue healthy cells. KM 9 d (E-H) Both apoptotic and necrotic signaling occurred in hair cells, but necrotic cell death seemed to predominate.

Conclusions

- Ototoxic drugs can destroy the sensory hair cells and supporting cells in the organ of Corti by either apoptosis or necrosis depending on the drug, dose and mode of treatment.
- In cochlear cultures treated for 48 h with 10 mM cisplatin, there was considerable hair cell death that was accompanied by signs of apoptotic and necrotic cell death in the supporting cells.
- In cochlear cultures treated with gentamicin for 24 there were signs of both apoptotic and necrotic cell signaling in both hair cells and supporting cells in the inner sulcus.
- In vivo, rats treated with KM/EA showed both apoptotic and necrotic cell death with necrotic labeling being the more common of the two.
- In vivo, rats treated for 9 d with KM, both necrotic and apoptotic labeling was present with apoptotic labeling being more prominent.

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